



Difficulties with the implemented xpert MTB/RIF for determining diagnosis of pulmonary and extrapulmonary tuberculosis in adults and children

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ABSTRACT

Background and aims: Handling of PTB and EPTB patients with adequate standard detection of MTBC and anti-TB drug sensitivity using accurate and rapid methods could provide good TB management and clinical treatment outcomes. The Xpert MTB/RIF assay is an automated, cartridge-based NAAT that can simultaneously detect MTBC and RIF resistance within 2 h. The aim of this study was to evaluate the implementation of Xpert for determining diagnosis of PTB and EPTB in adults and children.

Methods: A descriptive study was performed using e-TB Manager data from the MDR-TB Clinic at Dr. Soetomo Academic Hospital. Suspected TB cases were from the areas of East Java Province from January 2016 to December 2018. Xpert assay was conducted using standardized criteria for clinically suspected TB, and MTBC-positive results with RR were examined by the culture method using MGIT 960 BACTEC System.

Results: A total of 1181 (1181/3009, 39.25%) sputum samples from suspected new MDR-PTB cases tested positive for MTBC with 3.02% RR. Among 3893 sputum samples from previously treated probable MDR-PTB cases tested using Xpert, 1936 (49.73%) were MTBC positive with 13.20% RR. Among 59 new suspected MDR-PTB cases tested using MGIT 960 BACTEC System, 55 tested positive for MTBC, although all RR strains were highly sensitive to amikacin (100%), kanamycin (95%), and ofloxacin (89%). A total of 49 children with suspected PTB were tested using Xpert, revealing low positivity (12%) for MTBC, with all RR strains being rifampicin sensitive (RS). Of the 86 suspected EPTB cases tested using Xpert, very few were MTBC-positive (26%), with 91% RS.

Conclusions: This study revealed that in adults and children with PTB and EPTB, the Xpert assay achieved a low positivity detection rate for MTBC in samples from new or previously treated cases, and this could be the result of many factors.

1. Introduction

Tuberculosis (TB) is a chronic disease with a unique pathogenesis based on the interaction of multiple factors in the host, mycobacteria species or strain virulence, and environmental factors. In recent years, almost all countries have reported instances of TB and its problems, including morbidity, sequelae involving abnormal lung function, and mortality [1–5]. Tuberculosis in the form of Pulmonary TB (PTB) and extrapulmonary TB (EPTB), which mainly affect the adult population, has been shown to increasingly affect children [6–9].

This chronic disease, which is mainly airborne and affects the lung tissues, can spread to other organ tissues, known as EPTB, with a wide

spectrum of severity. This can include TB meningitis or spinal TB, diseases that can also exacerbate the appearance of, for example, lymphadenitis TB [9–11].

The crucial problem of PTB is its ability to spread through the air to the community and the surrounding environment. In the face of the urgency of challenges of PTB, various strategies must be immediately and intensively implemented to decrease or eradicate instances of TB [5–9]. Strategic activities to further this aim include promotion, preventive actions, early detection, effective diagnosis, rapid treatment, and rehabilitation [3,4].

Handling of PTB/EPTB patients with adequate and standard detection of *Mycobacterium tuberculosis* complex (MTBC) and identification of

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anti-TB drug sensitivity using accurate and rapid methods could provide good TB management and clinical treatment outcomes. The problem of TB is intensifying with the emergence of multi drug resistant (MDR) TB, mainly in the adult population, however, it is also an increasing problem in children.

The Xpert *Mycobacterium tuberculosis* (MTB)/Rifampicin (RIF) assay (Cepheid, Sunnyvale, CA) is a novel, automated, cartridge-based nucleic acid amplification test (NAAT) that can simultaneously detect MTBC and RIF resistance within 2 h. The assay is performed on the Cepheid GeneXpert multi-disease instrument system, which integrates sample purification, nucleic acid amplification, and target sequence detection. The Xpert MTB/RIF uses hemi-nested real-time PCR for the detection of MTBC and RIF-resistant (RR) strains using three primers to amplify the MTBC-specific sequence of the *rpoB* gene and five molecular probes to detect mutations within the RIF resistance-determining region (RRDR) of the gene. The assay can be performed directly on raw sputum or concentrated sediments. Samples are liquefied and deactivated by a mycobactericidal sample reagent, and after cartridge loading, all steps are fully automated and self-contained [2,6,7,12–16].

The purpose of this study was to evaluate the performance of the Xpert system in diagnosing PTB and EPTB in adults and children.

2. Methods

The descriptive study was carried out using e-TB Manager data from the MDR-TB Clinic at Dr. Soetomo Academic Hospital tertiary referral hospital in Indonesia. The suspected TB cases were from the area of East Java Province from January 2016 to December 2018. According to standardized criteria, clinically suspected TB patients were screened using the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA), and then MTBC and RR-positive results were examined using a culture method with the BACTEC MGIT 960 System (BD Diagnostic, USA) to confirm MTBC and to identify the first-line anti-TB sensitivity to rifampicin (R), isoniazid (H), streptomycin (S), and ethambutol (E) as well as to any second-line anti-TB drugs, specifically amikacin (Amk), kanamycin (Km), and ofloxacin (Of). This study was approved by the Ethics Committee in Health Research of Dr. Soetomo Academic Hospital, Surabaya, Indonesia (no. 618/Panke.KKE/X/2017).

3. Results

A total of 1181 (1181/3009, 39.25%) sputum samples from suspected new MDR-PTB cases tested positive for MTBC with 3.02% RR (Table 1). Among 3893 sputum samples from previously treated suspected MDR-PTB cases tested using Xpert, 1936 (49.73%) tested positive for MTBC with 13.20% RR (Table 2).

Regarding the positivity detection rate of Xpert, 51 of the 59 (86.44%) new suspected MDR-PTB cases (Table 3) and 322 of the 402 (80.09%) previously treated suspected MDR-PTB cases (Table 4.) tested positive for MTBC.

Among 59 new suspected MDR-PTB cases tested using the MGIT 960 system, 55 tested positive for MTBC, 42 (76.36%) showed RR, and all showed high sensitivity to amikacin (100%), kanamycin (95%), and ofloxacin (89%) (Table 5). Among 402 previously treated suspected MDR-PTB cases tested using, 384 tested MTBC-positive according to the

Table 1
MTBC positivity and RR tested in sputum samples from new suspected MDR-PTB cases using Xpert MTB/RIF during 2016–2018.

Total sputum samples	Xpert MTB/RIF
3009	MTBC Pos (1181/3009, 39.25%) RS 1090/3009 (36.22%) RR 91/3009 (3.02%)

RS: Rifampicin sensitive; RR: Rifampicin resistant.

Table 2
MTBC positivity and RR tested in sputum samples from previously treated suspected MDR-PTB cases using Xpert MTB/RIF during 2016–2018.

Total sputum samples	Xpert MTB/RIF
3893	MTBC Pos (1936/3893, 49.73%) RS 1422/3893 (36.53%) RR 514/3893 (13.20%)

RS: Rifampicin sensitive; RR: Rifampicin resistant.

Table 3
Concordance of Xpert MTB/RIF with the MGIT 960 system as a reference standard for new suspected MDR-PTB cases during 2016–2018.

Accuracy tested: Xpert	Reference standard: MGIT 960 System		Total
	MTBC ⁺	MTBC ⁻	
MTBC ⁺	51	4	55
MTBC ⁻	4	0	4
Total	55	4	59

Table 4
Concordance of Xpert MTB/RIF with the MGIT 960 system as the reference standard for previously treated suspected MDR-PTB cases during 2016–2018.

Accuracy tested: Xpert	Reference standard: MGIT 960 System		Total
	MTBC ⁺	MTBC ⁻	
MTBC ⁺	322	62	384
MTBC ⁻	14	4	18
Total	336	66	402

Xpert system, with 95% RR, while 336 tested MTBC-positive according to MGIT 960, with 88% RR; all strains showed high sensitivity to amikacin (100%), kanamycin (97%), and ofloxacin (90%) (Table 6).

Among children with suspected PTB, 49 tested by Xpert revealed low positivity (12%) for MTBC, with all strains being RS (Table 7).

A total of 86 samples from suspected EPTB cases tested by Xpert revealed a low MTBC positivity (26%), with 91% RS (Table 8).

4. Discussion

In this study, a low rate of MTBC detection was found using the Xpert MTB/RIF system among new and previously treated cases of adult PTB with suspected MDR-TB. In these cases, the Xpert MTB/RIF system provided low sensitivity in detecting MTBC in sputum samples.

The Xpert MTB/RIF has been approved as a rapid method for sputum examination to determine the presence of MTBC. The low sensitivity found in this study could be related to a low bacterial load in the sputum samples, which may be below the detection threshold of Xpert MTB/RIF. There may also have been PCR inhibitor substances present in the samples [9]. The low bacterial load in sputum samples could also be related to the use of anti-TB medication as it was difficult to obtain accurate data history. The low sensitivity of NAATs in sputum samples could again be related to the presence of PCR-inhibiting substances, with more substances being present in purulent sputum [9], requiring intensive processing of the sputum and concentrating of the samples before conducting the Xpert MTB/RIF assay.

Extensive research and meta-analyses involving Xpert MTB/RIF assay have been performed related to sputum respiratory samples from adults with PTB, demonstrating excellent accuracy, high sensitivity, specificity, and high positive and negative prediction values, reporting more than 95% [2,6,7,13–15,17] detection rate of MTBC and RIF resistance. This is better than the case-detection rate of acid-fast bacillus (AFB) smear microscopy, and the technique also allows to differentiate MTBC from nontuberculous mycobacteria [2,11,14,15].

A laboratory testing technique and algorithm needs to be

Table 5
Results of sputum samples from new suspected MDR-PTB cases tested using Xpert MTB/RIF and MGIT 960 System during 2016–2018.

Total sputum samples	Xpert MTB/RIF	MGIT 960
59	MTBC Pos (55/59, 93.22%) RS 0/55 (0%)	MTBC Pos (55/59, 93.22%) RR 55/55 (100%) RS 13/55 (23.63%) HS 25/55 (45.45%) SS 48/55 (87.27%) ES 42/55 (76.36%) AmkS 55/55 (100%) KmS 52/55 (94.54%) OfIS 49/55 (89.09%)

RS: Rifampicin sensitive; RR: Rifampicin resistant; HS: Isoniazid sensitive; SS: Streptomycin sensitive; ES: Ethambutol sensitive; AmkS: Amikacin sensitive; KmS: Kanamycin sensitive OfIS: Ofloxacin sensitive.

Table 6
Results of sputum samples from previously treated suspected MDR-PTB cases tested using Xpert MTB/RIF and MGIT 960 System during 2016–2018.

Total sputum samples	Xpert MTB/RIF	MGIT 960
402	MTBC Pos (384/402, 95.52%) RS 2/384 (0.52%)	MTBC Pos (336/402, 83.58%) RR 366/384 (95.31%) RS 41/336 (12.20%) HS 71/336 (21.13%) SS 269/336 (80.05%) ES 241/336 (71.72%) AmkS 336/336 (100%) KmS 326/336 (97.02%) OfIS 302/336 (89.88)

RS: Rifampicin sensitive; RR: Rifampicin resistant; HS: Isoniazid sensitive; SS: Streptomycin sensitive; ES: Ethambutol sensitive; AmkS: Amikacin sensitive; KmS: Kanamycin sensitive OfIS: Ofloxacin sensitive.

Table 7

Results of sputum samples from suspected TB children in East Jav tested using Xpert MTB/RIF during 2016–2018.

Total sputum samples	Xpert MTB/RIF	
49	MTBC Pos (6/49, 12.24%) RS 6/6 (100%)	RR 0

RS: Rifampicin sensitive; RR: Rifampicin resistant.

Table 8

Results of sputum samples from EPTB children in East Java tested using Xpert MTB/RIF during 2016–2018.

Total sputum samples	Xpert MTB/RIF	
86	MTBC Pos (22/86, 26.53%) RS 20/22 (90.90%)	RR 2/22 (9.09%)

RS: Rifampicin sensitive; RR: Rifampicin resistant.

established for the diagnosis of MTBC, using rapid NAAT methods, Xpert MTB/RIF, AFB microscopy, or rapid culture methods [10,18]. Based on the findings of this study, among clinically suspected MDR-TB patients, 3% of the new cases and 13% of the previously treated cases, showed RR. This indicates that the technique could be recommended as part of TB patient management policy on a per-case basis to determine RR or RS strains and make appropriate decisions to select anti-TB medication to achieve better clinical outcomes. The low RR detection rate of Xpert MTB/RIF requires further investigation regarding additional mutations of the RRDR of the *rpoB* gene [19].

In this study, regarding the evaluation of the specificity of Xpert compared with that of MGIT 960 system as a reference method, the concordance was 80%. The remaining 20% could be the result of false positives from Xpert, which was related to anti-TB-treated patients with non-viable bacteria and the system's DNA detection limit. The accuracy could not be fully analyzed in this study because not all cases were tested using both Xpert and MGIT 960 as a reference method.

The local policy in Indonesian hospitals to date has been to conduct laboratory examination using Xpert MTB/RIF when positive MTBC with RR results are confirmed using the BACTEC MGIT 960 System for identifying the anti-TB sensitivity profile of the first-line anti-TB drugs, rifampicin, isoniazid, streptomycin, and ethambutol as well as the second-line anti-TB drugs amikacin, kanamycin, and ofloxacin.

Of the 59 suspected new cases of RR-positive PTB MDR-TB, MGIT 960 analysis confirmed 76% RR. Fortunately, these strains were highly sensitive to amikacin (100%), manumycin (95%), and ofloxacin (89%). Furthermore, among 402 probable MDR-TB cases in previously treated patients, MGIT 960 analysis confirmed 88% RR, with high sensitivity to amikacin (100%), kanamycin (97%), and ofloxacin (90%). These findings could be used in recommendations for updating policy regarding TB management in Indonesia.

Regarding the performance of Xpert for detecting TB in children, this study examined a small number of cases using the Xpert MTB/RIF between 2016 and 2018. In 49 samples, there was a low positive detection rate for MTBC (12%), but all strains were sensitive to rifampicin.

There can be problems obtaining respiratory tract samples from children with TB, including difficulty in obtaining sputum and sputum samples that are paucibacillary [6,8]. There is a need for future studies regarding the performance of Xpert for detecting TB in children as well as for improving specimen-collection procedures, including expectorated sputum, gastric aspirate, blood or stool samples [1,8,20].

The problems of paucibacillary samples are evident in various samples from EPTB and TB-HIV patients [15,16]. In this study, we reported 86 cases of EPTB from 2016 to 2018, with 26% testing MTBC-

positive using Xpert MTB/RIF. Almost all samples were fine needle aspiration biopsy and cerebrospinal fluid samples. In EPTB cases, 90% of strains were sensitive to rifampicin.

In TB patient management policy in Indonesia, the use of Xpert MTB/RIF for pleurisy samples is not indicated because of its low performance, as many studies have reported [21–24], and the need for additional samples, i.e. blood and tissue biopsy, which is cumbersome and invasive [1,21,22,24,25].

In EPTB, there is a need for more than one specimen, either tissue biopsy, aspirate, or blood, and paucibacillary samples need to be concentrated through processing [10,11,21]. Aside from observations of clinical manifestation, the recommended policy for determining TB diagnosis, treatment, and monitoring includes the detection of surrogate biomarkers and viable bacilli as tools to evaluate clinical outcomes.

5. Conclusions

This study revealed that Xpert has low positivity for suspected new and previously treated cases of MDR-PTB. There was high concordance with the MGIT 960 system for MTBC detection, but discordance for RR detection. For children with suspected PTB and EPTB, Xpert showed low MTBC positivity detection. This could be caused by many factors. Criteria for decisions on clinical suspected PTB, EPTB, and MDR-TB in adults and children are variable, and accurate specimen processing and interpretation as well as consistent methods are required. Further study is needed on the prospective observation of all such clinical factors affecting clinical treatment outcomes.

Consent

This study was approved by the Ethics Committee in Health Research of Dr. Soetomo Academic Hospital, Surabaya, Indonesia (no. 618/Panke.KKE/X/2017).

Author contribution

NMM, SS, TK were the one who designed and conceive the protocol. NMM, SS, TK, EBK, DK, KK, TS, ZN, HC performed the data collection. ZN and HC were conducted the data analysis. All authors read and contributed to this work.

Declaration of Competing Interest

None.

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